EXPERIMENTAL STUDIES ON FAT METABOLISM WITH A BLOCKED RETICULOENDOTHELIAL SYSTEM

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EXPERIMENTAL STUDIES ON FAT METABOLISM WITH A BLOCKED RETICULOENDOTHELIAL SYSTEM

by

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I. INTRODUCTION

In 1949 a fat emulsion, which consisted principally of neutral fat and which could be administered intravenously, was produced by HIKASA in our laboratory. Soon after this, evidence was obtained histo- and biochemically by ASADA, NAKATA, NISHINO, SENO and OSA et al. that the infused fat globules which were first phagocytized by the cells of the reticuloendothelial system (R. E. S.) in the lungs, liver and spleen, were changed into phospholipides in these cells, and they were then oxidized completely to carbon dioxide and water after the phospholipides entered in the form of lipoproteins into the body cells such as the hepatic parenchymatous cells etc..

Therefore, if the cells of the R. E. S., in which the infused fat was first disposed of, can be blocked by some method, it can be expected that the subsequent fat metabolism will be disturbed. The present study was undertaken for the purpose of acertaining this point experimentally and, at the same time, observing indirectly the role of the R. E. S. in fat metabolism.

II. CAN THE CELLS OF THE R. E. S. BE BLOCKED?

Many investigators have long attempted the blockage of the cells of the R. E. S. in order to observe the metabolism of this system and the consequent production of antibodies. However, it is generally accepted at present that a complete and permanent blockage of the R. E. S. is impossible employing existing experimental methods. However, the subject studied by these investigators included the entire reticuloendothelial cells of the whole body, including histiocytes. It is natural

that the lymphatic apparatus and the histiocytes not contained in the blood vessels can not be blocked completely by such methods as the intravenous administration of fine carbon particles etc.. On the other hand, the object studied in the present experiment is the reticuloendothelial cells in the lungs, liver and spleen, which are the first portals for fat metabolism, as already clarified by experimental data of our laboratory. They are within a narrower limit which excludes the cells of the lymphatic system from the ones of the R. E. S. in the narrow sense as described by ASCHOFF. It is, therefore, easier for an intravenously infused blocking agent to reach these cells. We consider that the cells of this R. E. S. can be blocked and paralysed to some degree by means of an intravenous administration of a blocking agent.

III. EXPERIMENTAL MATERIALS

1) Blocking Agent……In such an experiment, fine carbon particles and colloidal silver etc. have long been employed. In the present experiment a 5 per cent suspension of finely divided carbon particles (Baikaboku, Kobaien made) was used after it was filtered twice and heated for sterilization.

2) Fat Emulsion…… A fat emulsion alone was used, containing 15 per cent sesame oil and 7 per cent glucose. The standard dose for intravenous administration of the fat emulsion was defined as 3.3 cc of 15 per cent fat emulsion, which is equal to 0.5 g fat per kg body weight.

3) Experimental Animals……Use was made of healthy male albino rabbits, ranging in weight from 2.0 to 2.5 kg. They had been kept on a standard diet for more than a week at least in order to stabilize the body weight and their general condition, and were fasted for 24 hours prior to the experiment.

IV. EXPERIMENTAL METHODS

1) Procedures for the Measurement of Total Fatty Acids……The determinations of the total fatty acids of serum, feces and urine were made by the method A of *Van de Kammer*. The total fatty acids of the organs were measured on the isolated fat from the organs by the method of BLOOR.

2) Procedures for the Measurement of Phospholipides……The phospholipides of the organs were determined by using an electrophotometer on the residue excluded acid soluble phosphorus from the organs by the FAWAZ-LIEB-ZACHERL method.

3) The Determination of Ketone Bodies……The method of GREENBERG and LESTER was adopted.

4) The Fat Staining for Histopathological Examination …… A modification of the method of GOLDMANN with Sudan III was used.

V. RESULTS

For the purpose of blocking the R. E. S., a large amount of the blocking agent must be intravenously infused as rapidly as possible. Excessive infusion, however, may cause death of the animals, and if some animals survive, it is probable that there is no constant blocking effect, since animals show significant individual differences in the R. E. S. function. In the present investigation, also, analogous obser-

vations of the rabbits were made, judging that function by the iris capillary fading test of TOKUMITSU.

Since the period when the black iris capillary color, after the final infusion of the carbon particles for the blockage had faded, was regarded as the period when all the intravenously infused carbon particles had left the blood stream in consequence of being completely phagocytized by the cells of the R. E. S., rabbits were intravenously injected with a standard dose of the fat emulsion.

1. In the Case of Blockage by the Administration of the Blocking Agent for 15 Consecutive Days (Group A).

According to the usual blocking method of the R. E. S., the rabbits are intravenously injected once daily for 15 successive days with the 5 per cent suspension of finely divided carbon particles at varying rates (initially 1 cc per kg body weight per diem, gradually increasing, terminally about 4 cc, to make up a total of about 40 cc per kg.). Thereafter, a standard dose of the fat emulsion was intravenously infused into the rabbits thus prepared. At definite intervals, these rabbits were sacrificed for the determination of total fatty acids contained in sera, lungs, liver and spleen. The controls, having received intravenously a standard dose of the fat emulsion, were determined in the same way.

The results are shown in Tables Ⅰ, Ⅱ, Ⅲ and Ⅳ Figs. 1, 2, 3, and 4. That is to say, changes in the total fatty acid contents of sera and of each organ of the blocked rabbits following intravenous infusion of the fat emulsion, showed almost no significant differences from the controls.

It is generally known that cells of such strong phagocytic ability as those of the R. E. S. have a powerful regenerative ability.

In the present experiment, when the organ weight of the blocked animals was divided by the organ weight of the control animals, the value was larger than that derived from Group C using a similar mode of calculation, despite equal amounts of carbon particles being infused into both.

That is, average values of 5.4 in the spleen, 2.0 in the liver and 0.3 in the lungs, were presented. These facts suggested that thus blocked animals come to

Table Ⅰ. Total Fatty Acid Concentrations of Serum Following Intravenous Infusion of Fat Emulsion.

	Control		Group A		Group B		Group C	
Time (hrs.)	Mean (g. %)	Change (%)	Mean (g. %)	Change (%)	Mean (g. %)	Change (%)	Mean (g. %)	Change (%)
0	0.22	0	0.23	0	0.31	0	0.28	0
1/6	0.43	+95	0.42	+83	0.60	+94	0.57	+103
1/2	0.26	+18	0.22	− 4	0.43	+40	0.46	+ 63
1	0.24	+ 9	0.24	+ 4	0.38	+24	0.40	+ 44
3	0.20	− 9	0.24	+ 4	0.35	+14	0.31	+ 10
6	0.21	− 5			0.29	− 8	0.29	+ 4
12	0.25	+13			0.32	+ 3	0.29	+ 1
24	0.24	+ 8			0.34	+10	0.34	+ 22

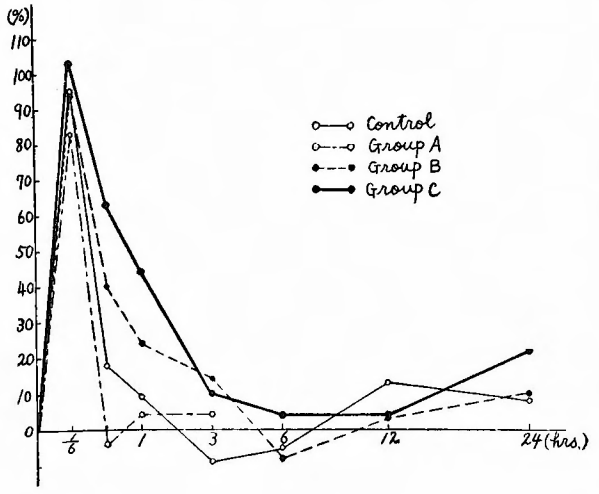
Table Ⅱ. Total Fatty Acid Contents of Lung Following Intravenous Infusion of Fat Emulsion.

	Control		Group A		Group B		Group C	
Time (hrs.)	Mean (g. %)	Change (%)	Mean (g. %)	Change (%)	Mean (g. %)	Change (%)	Mean (g. %)	Change (%)
0	2.27	0	2.18	0	2.40	0	2.24	0
1/6	2.54	+11	2.25	+ 3	2.59	+ 8	2.91	+30
1/2	3.07	+39	2.89	+33	3.12	+30	2.87	+28
1	2.47	+ 9	2.36	+ 9	2.86	+19	2.93	+31
3	2.14	− 6	2.61	+19	2.45	+ 2	2.49	+11
6	2.39	+ 5			2.26	− 6	2.40	+ 7
12	2.34	+ 3			2.67	+11	2.35	+ 5
24	2.17	− 4			2.57	+ 7	1.93	−14

Table Ⅲ. Total Fatty Acid Contents of Liver Following Intravenous Infusion of Fat Emulsion.

	Control		Group A		Group B		Group C	
Time (hrs.)	Mean (g. %)	Change (%)	Mean (g. %)	Change (%)	Mean (g. %)	Change (%)	Mean (g. %)	Change (%)
0	3.43	0	3.11	0	3.22	0	2.91	0
1/6	4.29	+25	3.73	+20	3.72	+16	3.29	+13
1/2	5.52	+61	4.76	+53	4.14	+29	3.06	+ 5
1	5.15	+50	4.70	+51	3.69	+15	3.49	+20
3	4.08	+19	3.36	+ 8	4.05	+26	3.58	+23
6	3.67	+ 7			3.73	+16	3.23	+11
12	3.27	− 5			3.55	+10	3.52	+21
24	3.62	+ 6			3.03	− 6	3.23	+11

Fig. 1. Changes in Total Fatty Acid Concentrations of Serum Following Intravenous Infusion of Fat Emulsion.



have the ability to dispose of fat to the same degree that the non-blocked animals have, by reason of a powerful regeneration and proliferation of the reticuloendothelial cells in the former animals, when the blocking agent, the suspension of carbon particles, is infused intravenously in the animals for such a long period of time. It was, subsequently, found that it is impossible to achieve our purpose by means of such a slow blocking method.

2. In the Case of Blockage by the Administration of the

Fig. 2. Changes in Total Fatty Acid Contents of Lung Following Intravenous Infusion of Fat Emulsion.

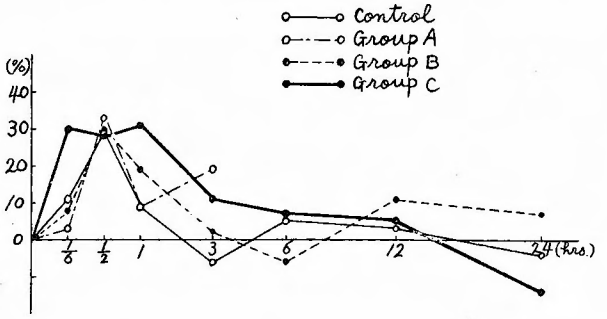


Fig. 3. Changes in Total Fatty Acid Contents of Liver Following Intravenous Infusion of Fat Emulsion.

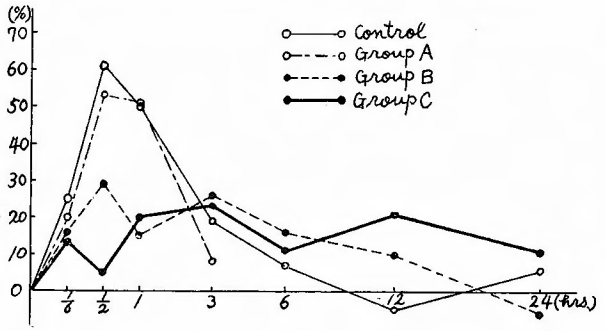
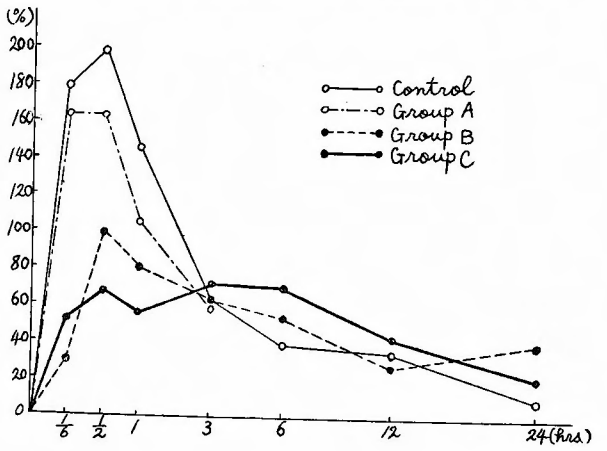


Fig. 4. Changes in Total Fatty Acid Contents of Spleen Following Intravenous Infusion of Fat Emulsion.



Blocking Agent for 5 Consecutive Days (Group B).

The 5 per cent suspension of carbon particles was introduced by the intravenous route into the rabbits at a rate of 8 cc per kg body weight per diem, divided into 2 doses, morning and evening, to achieve a total of 40 cc per kg body weight for 5 consecutive days. Furthermore, after injected intravenously with the standard dose of the fat emulsion, the rabbits were sacrificed at definite intervals for the determination of total fatty acids in sera, lungs, liver and spleen, and of phospholipides in the organs.

The total fatty acid concentrations of sera, as shown in Table Ⅰ and Fig. 1, became as high as those of the controls 10 minutes after infusion of the fat emulsion and it returned slowly to the original value 3 hours after infusion. The total fatty acid contents of lungs, as is noticed in Table Ⅱ and Fig. 2, presented almost no significant differences from the controls, but when they were observed in detail, they showed a tendency to delay slightly those decreasing in contrast with the controls. This finding, similarly to the results obtained by KIMURA, may indicate that the lungs of rabbits are difficult to block by means of the intravenous administration of a suspension of carbon particles. The results of the total fatty acid contents of livers were recorded in Table Ⅲ and Fig. 3; the contents showed a remarkably lower peak than that of the controls 30 minutes after infusion, thereafter the value decreased gradually,

maintaining, on the contrary, higher values than the controls 12 hours after infusion. A tendency similar to this was also obtained for the spleen. With respect to the phospholipide contents of the organs, those of the lungs began to increase slightly later than the controls as shown in Table V and Fig. 5. The increase of those in the liver and spleen appeared much later and was much lower in comparision to the controls (Table VI, VII and Fig. 6, 7).

From these investigations, it would be reasonable to consider that; since the function of the reticuloendothelial cells participating in fat metabolism, was somewhat blocked and paralysed by the intravenous administration of the carbon particles, the ability of phagocytizing the infused fat by the reticuloendothelial cells was reduced. Therefore, the time that the infused fat was in the blood stream was prolonged and at the same time, the changing action into phospholipides from neutral fat in the cells of the R. E. S. was disturbed.

3. In the Case of Blockage by the Administration of the Blocking Agent for 16 Consecutive Hours (Group C).

Table IV. Total Fatty Acid Contents of Spleen Following Intravenous Infusion of Fat Emulsion.

	Control		Group A		Group B		Group C	
Time (hrs.)	Mean (g. %)	Change (%)	Mean (g. %)	Change (%)	Mean (g. %)	Change (%)	Mean (g. %)	Change (%)
0	1.46	0	1.39	0	1.61	0	1.30	0
1/6	4.07	+179	3.66	+163	2.30	+30	1.98	+52
1/2	4.35	+198	3.65	+163	3.20	+99	2.17	+67
1	3.58	+145	2.84	+105	2.89	+80	2.03	+56
3	2.38	+ 63	2.19	+ 58	2.62	+63	2.24	+72
6	2.03	+ 39			2.48	+54	2.21	+70
12	1.97	+ 35			2.06	+28	1.86	+43
24	1.60	+ 10			2.26	+40	1.59	+22

Table V. Phospholipide Contents of Lung Following Intravenous Infusion of Fat Emulsion.

	Control		Group B		Group C	
Time (hrs.)	Mean (g. %)	Change (%)	Mean (g. %)	Change (%)	Mean (g. %)	Change (%)
0	1.93	0	1.80	0	1.72	0
1/6	1.91	− 1	1.72	− 5	1.77	+ 3
1/2	2.18	+13	1.86	+ 3	1.62	− 6
1	2.30	+19	2.09	+16	1.79	+ 4
3	2.09	+ 8	1.99	+10	1.89	+10
6	1.85	− 4	1.68	− 9	1.89	+10
12	1.64	−15	1.82	+ 1	1.65	− 4
24	1.88	− 2	1.54	−15	1.79	+ 4

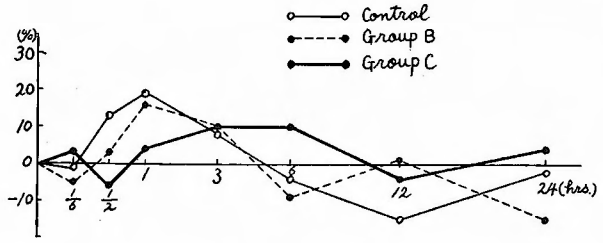
Table Ⅵ. Phospholipide Contents of Liver Following Intravenous Infusion of Fat Emulsion.

	Control		Group B		Group C	
Time (hrs.)	Mean (g. %)	Change (%)	Mean (g. %)	Change (%)	Mean (g. %)	Change (%)
0	2.74	0	2.51	0	2.43	0
1/6	2.88	+ 5	2.48	− 1	2.28	− 6
1/2	2.68	− 2	2.77	+10	2.53	+ 4
1	2.81	+ 3	2.24	−10	2.60	+ 7
3	3.33	+22	2.51	0	2.55	+ 5
6	3.50	+28	2.48	− 1	2.16	−11
12	2.99	+ 9	2.96	+18	2.77	+14
24	2.87	+ 5	2.38	− 5	2.67	+10

Table Ⅶ. Phospholipide Contents of Spleen Following Intravenous Infusion of Fat Emulsion.

	Control		Group B		Group C	
Time (hrs.)	Mean (g. %)	Change (%)	Mean (g. %)	Change (%)	Mean (g. %)	Change (%)
0	1.15	0	1.08	0	1.02	0
1/6	1.29	+12	1.13	+ 5	0.96	− 6
1/2	1.03	−10	1.17	+ 8	0.95	− 7
1	1.49	+29	0.95	−12	1.10	+ 8
3	1.25	+ 9	1.23	+14	0.98	− 4
6	1.82	+58	1.21	+12	1.09	+ 7
12	1.47	+30	1.46	+34	1.21	+19
24	1.27	+10	1.20	+11	1.17	+15

Fig. 5. Changes in Phospholipide Contents of Lung Following Intravenous Infusion of Fat Emulsion.



It was well demonstrated by the above mentioned experiments that, for the purpose of blocking the reticuloendothelial cells, a large amount of the carbon particle suspension should be intravenously administered in a short period of time so that there would be no regeneration or proliferation of these cells. This led the present author to carry out the next investigation.

The rabbits were intravenously injected at intervals of 2 hours for 16 successive hours with the suspension of carbon particles, about 40 cc per kg body weight in total. After having received intravenously the standard dose of the fat emulsion by an identical method as described above, the blocked rabbits were sacrificed at definite intervals for the measurement of total fatty acids in sera, lungs, liver and spleen, and for the phospholipides in these organs.

The fluctuations in the total fatty acid concentrations of sera were exhibited

in Table I and Fig. 1; the peak was higher than that of Group B 10 minutes after infusion of the fat emulsion. The concentrations attained still higher values than Group B one hour after infusion, returning to the original value 3 hours after infusion. As shown in Table Ⅱ and Fig. 2, the total fatty acid contents of the lungs, 10 minutes after infusion, reached their maximum and this continued for one hour, decreasing thereafter, but were still higher than Group B 3 hours after infusion. Increase in the total fatty acid contents of liver and spleen was remarkably less than in the control, Groups A or B. Furthermore, reduction therein was much slower. The graphed fluctuations were found, as a whole, to be flat patterns (Table Ⅲ, Ⅳ and Fig. 3. 4). It was elucidated from histopathological study on these rabbit organs that these results were not dependent upon the emboli of carbon particles or of the infused fat globules in the capillaries. The phospholipide contents of the lungs, as shown in Table V and Fig. 5, increased slightly after 3 to 6 hours but the increase manifested itself markedly later than the controls. Twelve hours after infusion, the phospholipide contents of the liver and spleen reached their highest levels which were considerably lower than those of the controls (Table VI, VII and Fig. 6, 7).

Fig 6. Changes in Phospholipide Contents of Liver Following Intravenous Infusion of Fat Emulsion.

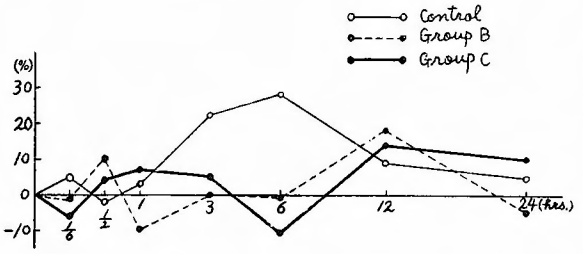
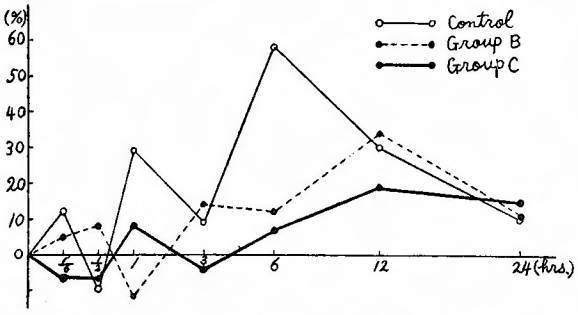


Fig. 7. Changes in Phospholipide Contents of Spleen Following Intravenous Infusion of Fat Emulsion.



Since the cells of the R. E. S. were relatively well blocked, the time that the infused fat remained in the blood stream was considerably prolonged and the maximum value in the total fatty acid content of liver and spleen was remarkably less than that of the controls, though it appeared 3 hours after infusion, at which time the infused fat should have left the blood stream. The disappearing time of the infused fat globules from the blood stream was approximately the same in Group B as well as in Group C which was blocked much more powerfully than the former. Therefore, those infused fat globules which are first disposed of in the organs other than the liver and spleen must be larger in quantity in Group C than Group B. Which organ could be performing such a function in fat metabolism? This organ was found to be the lung which was difficult to block, from the results which showed that an increase in the total fatty acid in the lungs appeared earlier and was maintained longer after infusion

in Group C than Group B. It is surmised that the ability to dispose of fat in the lung is compensatively accelerated instead of the liver and spleen in the case of blockage of the R. E. S.. Further, it should also be considered that the phospholipide contents of the organs in Group C suggest a difficulty in the phagocytosis of the fat globules by the reticuloendothelial cells and a disturbance in the changing action into phospholipides from neutral fat in these cells. However, it goes without saying that the R. E. S. can not be blocked completely for a long period of time even by such an intensive blocking method as used in Group C.

As mentioned above, it has already been illustrated by the investigators in our laboratory that; the infused fat globules are converted to phospholipides in the reticuloendothelial cells. Further, they are carried into the hepatic parenchymatous cells and other tissue cells in the form of lipoproteins. Afterwards, fatty acids contained in this fat emulsion break down completely into 2 carbon fragment viz. acetyl-coenzyme A (Acetyl-CoA) by successive β-oxidation. A part of Acetyl-CoA is oxidized completely to carbon dioxide and water in the tricarboxylic acid cycle (T. C. A. cycle), after entering directly into this cycle by condensation with oxaloacetic acid which is an intermediate product in the oxidative breakdown of carbohydrates. Pairs of the Acetyl-CoA, unable to enter into the T. C. A. cycle, react together chiefly in the liver to form ketone bodies, which diffuse into the blood stream and are carried to the extrahepatic tissues. In these tissues the ketone bodies are again converted to Acetyl-CoA and indirectly enter into the T. C. A. cycle.

Accordingly, when the fat emulsion is intravenously infused into the rabbits, an increase in the blood ketone body level should occur in these animals. In the present experiment, while a remarkable increase in the blood ketone body levels following an intravenous infusion of the fat emulsion, as described by HASHINO, was observed, in the non-blocked rabbits serving as controls, no increase following the infusion of equivalent doses of the emulsion could be noticed in such blocked rabbits as those of Group C (Table VIII and Fig 8.). These results indicate, more clearly, that the ability to dispose of fat in the R. E. S. is reduced markedly in Group C.

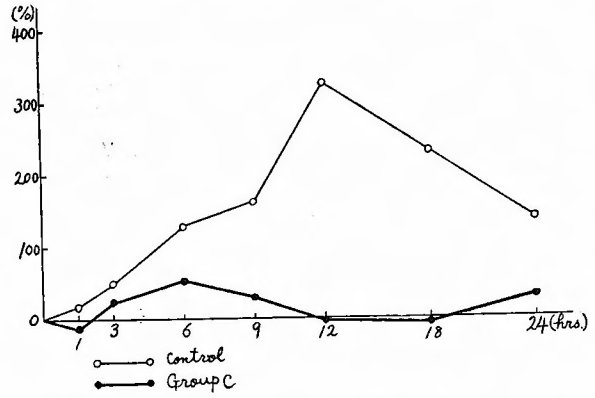
Table VIII. Blood Ketone Body Levels Following Intravenous Infusion of Fat Emulsion.

	Control		Group C	
Time (hrs.)	Mean (mg/dl)	Change (%)	Mean (mg/dl)	Change (%)
0	0.63	0	2.17	0
1	0.75	+ 19	1.89	− 13
3	0.94	+ 49	2.67	+ 23
6	1.43	+ 127	3.31	+ 53
9	1.64	+ 160	2.77	+ 28
12	2.67	+ 324	2.09	− 4
18	2.09	+ 232	2.01	− 7
24	1.50	+ 138	2.79	+ 29

VI. DISCUSSION

As mentioned above, when the reticuloendothelial cells, which are the first to dispose of an infused fat emulsion, have been intensively blocked by the intravenous administration of the carbon particles, the presence in the blood stream of the infused fat was considerably prolonged, the increase in the total fatty acid contents of the liver and spleen was remarkably

Fig. 8. Changes in Blood Ketone Body Levels Following Intravenous Infusion of Fat Emulsion.



slight, and that, their decrease thereafter was markedly gradual.

These facts indicate that the infused fat phagocytized by the reticuloendothelial cells of the liver and spleen is less in quantity and that the ability to dispose of the phagocytized fat by these cells viz., their function to change neutral fat into phosholipide, is disturbed; accordingly, the phagocytized fat is retained in these cells for a long period of time. The changes in the phospholipides of the liver and spleen represent quite clearly the disturbance of the fat metabolism in the R. E. S.. The infused fat emulsion, which can not be disposed of in the liver and spleen, is metabolized in the lung, whose ability to dispose of fat is enhanced compensatively by the reduction of the function of the R. E. S..

However, while the above mentioned facts were obtained only in Group C, in which the R. E. S. was powerfully blocked in a short period of time, no significant differences in the ability to dispose of the infused fat were found between the controls and Group A blocked by the slow blocking method.

Complete blockage of the cells of the R. E. S. is impossible and is well demonstrated by the fact that a small quantity of the infused fat is still metabolized by slow degrees in the liver and spleen in Group C and the ability to dipose of the infused fat is more expedited in the lung in that group than in the controls. Furthermore, following intravenous infusion of the fat emulsion, a remarkable increase in the blood ketone body levels was observed in the controls, while there was no increase in Group C. These findings not only suggest a disturbance of the metabolic process of the infused fat but also that a disharmony of various enzyme systems participating in the fat metabolism or the reduction of the constitutional activity is caused by the administration of the carbon particles.

It is demonstrated more clearly, but indirectly, by the foregoing results that the intravenously infused fat is utilized effectively in the living organism after being phagocytized by the cells of the R. E. S. and is then converted into a phospholipid in these cells. The fat can never be oxidized without these processes in the reticuloendothelial cells.

However, it is also necessary to consider whether a part of the infused fat may be carried to sites other than the lung, liver and spleen. First of all, excretion from the body of the infused fat comes into question. Though fat is, as is generally known, secreted from the sebaceous glands or the mammary glands as a component in sebum or milk respectively, these secretions may be disregarded

in the present experiment. ASADA pointed out the possibility that some of the fat was excreted with sputum through the trachea, because of the finding that the alveolar phagocytes which phagocytized fat globules after surplus infusion, frequently in the alveolar spaces separated from the alveolar wall. Accordingly, in Group C, in which the lung metabolizes larger amounts of fat in comparision to Group B and the controls, a part of the infused fat may be expected to be excreted with sputum. However, since an increase in the phospholipid content of the lung appeared clearly although delayed, the infused fat is regarded as being oxidized slowly in the lung. Therefore, any fat, excreted through such a pathway, would be negligible in quantity.

With regard to other excretory pathways, there is the excretion through the urinary and the digestive tracts. It has generally been known that phospholipides may occasionally be demonstrated in urine containing albumin, that urine obtained from nephrotic patients has cholesterin, and that many fat globules exist in the epithelium of the renal tubule. Therefore, it is possibile that infused fat may be excreted with urine. SPERRY and his co-workers verified that relatively large amounts of lipids are secreted into the small intestine. A considerable portion of this secretion is reabsorbed in the large intestine. The remainder, together with lipids secreted into the colon, are excreted with feces. From this point of view, it is considered that the infused fat is probably excreted with the feces.

The author carried out the following additional experiment. After the rabbits in Group C were injected intravenously with the standard dose of the fat emulsion, urine samples were collected from them for 24 hours. Then the total fatty acid content of the urine samples was measured. At the same time, healthy rabbits, reared on 500 g of cabbage daily, corresponding to 1.25 g of fat, were administered a suspension of carbon particles by a method similar to that of Group C, after the amount of feces excreted had become constant. After the standard dose of the fat emulsion was infused in these animals, the feces excreted were collected for 2 days and the total fatty acid content of the feces was determined. As controls, the total fatty acid content of the urine and in feces from non-treated rabbits and from rabbits given the fat emulsion alone were used. The results are presented *en bloc* in Table IX. That is, the infused

Table IX. Total Fat Contents of Feces and Urine after the Injection of Fat Emulsion.

		Feces			Urine	
	No. of Rabbit	Volume for 48 hrs.	Concentration	Amount Excreted for 24 hrs.	Volume for 24 hrs.	Amount Excreted for 24 hrs.
Control	H. 13	17.0g.	2.60%	0.22g.	170cc	0
	H. 14	15.0g.	3.43%	0.26g.	205cc	0
Fat Emulsion Alone	H. 13	19.0g.	2.97%	0.28g.	160cc	0
	H. 14	16.5g.	2.78%	0.23g.	190cc	0
Blockade and Fat Emulsion	H. 13	7.5g.	3.78%	0.14g.	130cc	0
	H. 14	5.5g.	3.96%	0.11g.	140cc	0

Diet daily, Cabbage 500g. (Fat Content 1.25g.)

fat can not be excreted in the urine or feces even in the blocked rabbits. Accordingly, the infused fat emulsion is hardly excreted directly from the body. Therefore, as to the fate of the infused fat emulsion, leakage of the fat globules out of the blood vessels must be considered. In fact, the present author

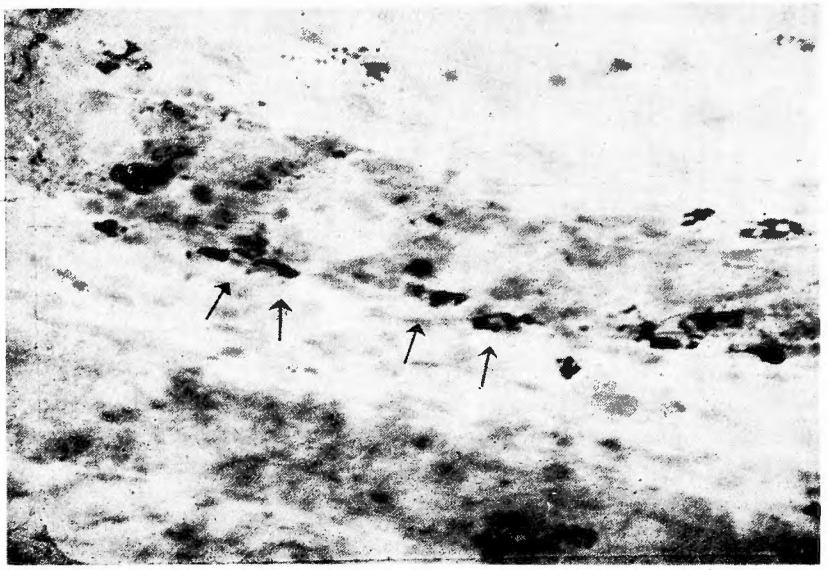


Fig. 9. Fat Globules under Adventitia Cells of Postcapillary Vein in Omentum 30 Minutes after Intravenous Infusion of Fat Emulsion. (Sudan Ⅲ)

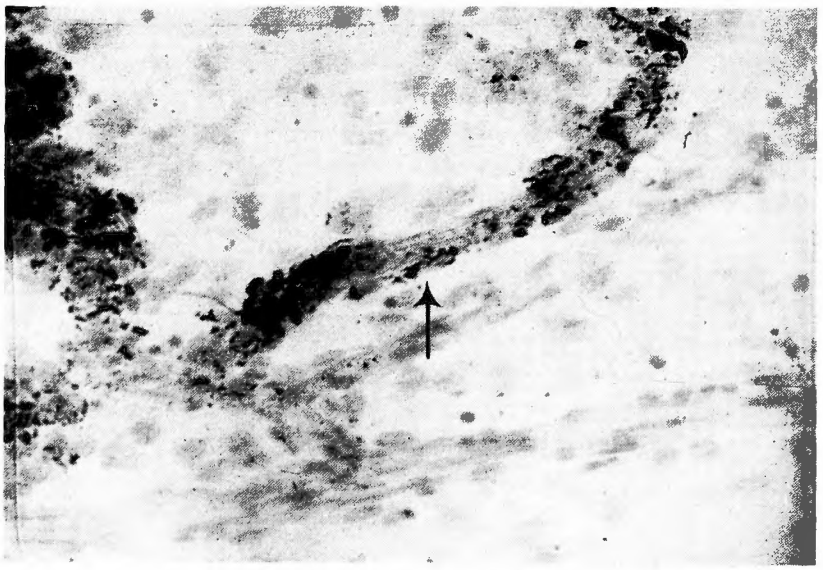


Fig. 10. Fat Globules under Adventitia Cells of Postcapillary Vein in Omentum 1 hour after Intravenous Infusion of Fat Emulsion. (Sudan Ⅲ)

observed black changed parts hither and thither in the omentum majus at the autopsy of the rabbits receiving large amounts of carbon particles. It was clarified by a histopathological examination that these black parts were attributed to the carbon particles which, as stated by AMANO, leaked out of the postcapillary veins.

Accordingly, it becomes natural to consider that the infused fat globules with a diameter less than 0.5 μ also will be able to leak out of the blood vessels. Thereupon, the following experiment was undertaken. The rabbits blocked by the Group C method were injected with a standard dose of the fat emulsion. Thereafter the animals were sacrificed at definite intervals. Extensive preparations of omentum were stained using the modification of the GOLDMANN method and was examined microscopically. As shown in Figs. 9 and 10, many fat globules that seem to be the infused fat were observed markedly under the adventitia cells of the postcapillary veins in the omentum. This finding was noticed 10 minutes to 3 hours after infusion, but not in the cases of more than 3 hours duration. These fat globules get out of the blood vessels as well as carbon particles, as emphasized by AMANO. There is slight fear that this finding may be ascribed to the removal of fat which existed physiologically in the omentum by alcohol etc. used in the staining manipulation. However, because a definite time relationship was observed between the appearance of these fat globules under the adventitia cells and the intravenous infusion of the fat globules, and because CHAIKOFF et al. demonstrated that after intravenous injection of tripalmitin emulsion labeled with C^{14} into mice, a part of the emulsion was found in the storage fat in a short period of time, there no doubt exists the possibility that some of the infused fat globules leak out of the blood vessels in the blocked rabbits. Further precise experiments with radioactive isotopes is required to clarify these points.

No fatty livers were observed histopathologically in the various blocked rabbits. Accordingly, as mentioned by ASADA, it could not be ascertained that surplus infused fat globules infiltrated directly into the hepatic parenchymatous cells.

Ⅶ. SUMMARY

Biochemical and histopathological studies were made, with the following results, on fat metabolism, using rabbits in which the R. E. S. had previously been blocked with carbon particles, and which were then intravenously infused with a fat emulsion produced in our laboratory.

1) From the features of fat metabolism, it was impossible that the R. E. S. was completely blocked for a long period of time by the intravenous administration of the carbon particles.

2) However, temporary blockage thereof was relatively complete.

3) By the blockage of the R. E. S., the metabolic process of the infused fat was markedly disturbed. That is, the infused fat, even when consisting of higher

fatty acids, were never metabolized without first being disposed of in the cells of the R. E. S. in the lungs, liver and spleen.

4) With regard to the excretion from the body of the infused fat, there is some possibility, of the excretion through the trachea and leakage out of the blood vessels. No excretion in urine or feces was found.

5) The ability to dispose of the infused fat in the lungs was compensatively enhanced when the disposal ability of the liver and spleen had been reduced.

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和 文 抄 録

網内系機能封鎖時に於ける脂肪代謝に関する実験的研究

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教室創製の脂肪乳剤を，予め墨粒を以て網内系機能を封鎖した家兎の静脈内へ注入した際の生体内脂肪代謝の様相を考究し，次の結論を得た．

1） 生体内脂肪代謝の様相からみると，墨粒の静脈内注入による網内系細胞の機能封鎖を完全且つ長期間に亘り行うことは不可能である．

2） 併し乍ら網内系細胞機能の封鎖目的が比較的完全という程度でよいならば，墨粒の短期間，而も大量の静脈内注入でよくその目的を達する．

3） 網内系機能封鎖によつて注入脂肪の処理は不円滑，且つ著しく遅延する．従つて注入脂肪がたとえ高級脂肪酸の Trigyceride からなる場合でも，先ず肺，肝，脾臓の網内系細胞群によつて一次的に処理されねば，之れがその後の生体内代謝過程は行われ得ない．

4） 網内系細胞の機能封鎖が行われていても注入脂肪の尿中，糞便中への排泄は毫も認められず，唯僅かに注入脂肪球の肺胞腔内への排泄と血管外漏出の可能性が考えられる．

5） 肺臓は墨粒の静脈内注入という方法によつては如何に之を強力に行つても封鎖され難い．而して肝，脾の網内系細胞の機能封鎖が比較的よく行われている際には肺臓の注入脂肪の処理能力は却つて代償的に亢進する．